

AMENDMENTS TO THE SPECIFICATION

After the title on page 1, please delete the "inventor identification" section in its entirety.

Insert the following new section on page 1 following the title:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a division of Application No. 10/021,985, filed December 13, 2001, which is a division of Application No. 09/326,501, filed June 4, 1999 (now U.S. Patent No. 6,388,113).

Amend Table 1 beginning at page 2, line 17, to read as follows:

Table 1

FATTY ACIDS	CARBON CHAIN	PERCENT WEIGHT
SATURATED ACIDS		
Palmitic	C16:0	5%-7% = (normal) 2%-4% 2%-5% = (high oleic) 22%-40% = (high palmitic)
Stearic	C18:0	< 10% but usually 3%-7% (normal, high oleic, high palmitic)
UNSATURATED ACIDS		
Oleic	C18:1	17%-20% <u>17%-30%</u> = (normal) 9%-12% = (high palmitic) 75%-90% = (high oleic)
Linoleic	C18:2	50%-70% = (normal high palmitic) (high oleic) = 2%-10%

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Amend the paragraph beginning at page 3, line 22, as follows:

The availability of other high oleic sunflower seed is listed ~~with within a number of~~ patent documents. For example, there is intellectual property describing high oleic lines which is believed to be based on the Russian Pervenets sunflowers in U.S. Patent No. 4,627,192 and Re-examination certificate B1 4,627,192, issued October 17, 1995, and U.S. Patent No. 4743,402 ~~4,743,402~~ and Re-examination certificate B1 4,743,402, issued April 8, 1997, to Flick. ~~This intellectual property lists These patents list~~ a number of sunflower varieties that are commercially available for breeding purposes that can be licensed under the Fick patents through a company called A.C. Humko. Additionally, the Fick U.S. ~~patent~~ Patent No. 4,627,192 indicates that oleic seeds of Sigco 41A, 41b, 853R, 4117b, 273W, ~~and~~ 416R are available from the Lubrizol Corporation, 29400 Lakewood Blvd., Wickliffe, Ohio (USA) 44092.

Amend the paragraph beginning at page 4, line 2, as follows:

Some of this type of research is outlined in *Osorio et al.*, in *Crop Sci.* 35:739-42 (1995). This article describes sunflower seeds developed by traditional breeding and mutagenesis to produce seeds with a high stearate content. This type of research is also outlined in PCT application number EP95/00369 which is entitled "Sunflower seeds and oil having a high stearic acid content". This application teaches, as its name implies, a sunflower oil with increased stearic acid content. One way to obtain this oil is by treating parent seed with a mutagenic agent to induce one or more mutations in the stearic acid biosynthesis pathway. This process resulted in an increased production of stearic acid in the sunflower oil in a range between 12% and up to 35% by weight of stearic acid related to the total amount of fatty acid in the oil. High stearic acid producing seeds discussed in this patent are under deposit in the American Type Culture Collection (ATCC). Sunflower seeds identified as "CAS-3" have a an average stearic acid content of 25% by weight, related to the total amount of fatty acids in the oil. These seeds were

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deposited on December 14, 1994, with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852, U.S.A., under deposit accession number ATCC 75968. ~~And sunflower~~ Sunflower seeds identified as "CAS-4" having an average stearic acid content of 15% by weight, related to the total amount of fatty acids in the oil, were deposited on December 14, 1994, with the American ~~type~~ Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852, U.S.A., under deposit accession number ATCC 75969.

Amend the paragraph beginning at page 4, line 21, as follows:

This application PCT/EP95/00369 suggests that oil from high stearic lines could be combined with oil from high oleic lines for certain industrial uses. Unfortunately, although this combination of two oils is useful in many instances, there remains a need for a seed that produces high levels of both stearic and oleic fatty acids in the same oil. ~~Particularly, particularly~~ since the levels of linoleic acid produced by these stearic acid lines may tend to produce a less desirable profile of fatty acids, ~~then than~~ would be produced by a hybrid producing high stearic, high oleic acid.

Amend the paragraph beginning at page 4, line 29, as follows:

Traditional breeding and mutagenesis has not been the only tool used to form seeds producing oil with different fatty acid profiles. Increases in stearic acid in oil bearing plants have also been addressed by the introduction of transgenes ~~in to~~ into the germplasm, to alter the fatty acid biosynthesis pathway of the vegetable oil. The fatty acid biosynthesis in vegetable oil, but more particularly sunflower oil, includes the biosynthesis of basically two saturates palmitate, stearate and two unsaturates oleate and linoleate. To give a simplified description of the biosynthesis pathway, it is sufficient to say, that palmitate (C16:0) is by enzymatic action chemically modified to form stearate (C18:0), which by enzymatic action is modified to produce oleate (C18:1), that is further modified to form linoleate (C18:2), some minor amounts of arachidic

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(C20:0) and behenic (C22:0) acids are also formed from stearate. In oilseeds the stearoyl-ACP desaturase is the enzymatic action which introduces the first double bond on stearoyl-ACP to form oleoyl-ACP. Thus, this is an enzyme that assists in the determination of the unsaturation in the C18 length fatty acids.

Amend the paragraph beginning at page 5, line 19, as follows:

Additionally, Pioneer Hi-Bred International, Inc., ~~have has~~ increased the levels of both stearic acid and palmitic acid in sunflowers ~~was~~ through the inhibition of the plant enzyme stearoyl-ACP desaturase. This research was surprising in light of other transformations in other plants that indicated that this enzyme would not ~~effect affect~~ palmitic acid levels only stearic acid levels. Unfortunately, palmitic oil is not viewed as being a healthy oil. This research is indicated in PCT/US97/01419.

Amend the paragraph beginning at page 11, line 24, as follows:

Therefore, vegetable oils are modified to form hard fats. These vegetable oils are hydrogenated and/or transesterified to increase the percentage of saturated fatty acids. However, the resulting oils are unfortunately not necessarily healthier fats. The hydrogenation process produces "trans fatty" acids that probably are even less healthy than saturated fatty acids. ~~While, while~~ the transesterification process randomly ~~changes exchanges~~ the fatty acid acids within the three positions. Thus, neither of the two chemical modifications works well to truly form healthy fats.

Amend the paragraph beginning at page 15, line 24, as follows:

This HOHT line is deposited with the ATCC as _____ at 10801 University Boulevard, Manassas, VA 20110-2209, on September 7, 1999, and assigned PTA-628. As Table 3 indicates, this line has more stearic acid at 15 days after flowering than the HOLT. This stearic acid level at 15 DAF can also be employed as a rough screening protocol for selecting

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HOHT lines. The relative affinity of the enzymes over the stearoyl-ACP standardised with respect to the one over oleoyl-ACP are also shown in Figure 2.

Amend the paragraph beginning at page 16, line 6, as follows:

Commercially and publicly available high oleic material, the deposited material or newly formed mutated high oleic lines produced according to the examples or by other methods of mutation or transformation can be screened according to the procedure outlined in example 6 4. The selected lines can have an increased thioesterase Vmax on the substrate stearoyl-ACP at 15 DAF having activity levels as indicated in the definition of high levels of thioesterase. The result of finding a HOHT line is that the fatty acid content will most often evidence an increase in the stearic acid level (when compared to a HOLT) in the line while maintaining a high oleic content. It should be noted that the oleic content of these (HOHT) lines is more likely to be in the sixty, seventy or low eighty range versus the high eighties.

Amend the paragraph beginning at page 26, line 28, as follows:

EXAMPLE 3

Sunflower plants were grown from the sunflower seeds of the HOHT line shown in Table 4. Sunflower plants were also grown from the sunflower seeds of CAS-3. The lines were crossed. The plants were assisted by artificially artificial pollination in order to ensure adequate seed production occurred. The F1 seed was produced on the HOHT line, or vice versa, and harvested. The F2 seeds with more than 20% stearate and more than 40% oleate were selected. Although this produces the oil of the present invention the level of production is limited. Therefore fixed inbred lines evidencing seeds with these oil profiles are desirable. These homozygous fixed inbred HSHO lines can then be crossed to form hybrid seed, which will produce F2 seed evidencing the desired oil traits of the present invention. Toward this end the F1 seeds were planted and produced plants were selfed in isolated conditions and F2 seed was

produced. The F2 seed was tested for the three traits, high stearic, high oleic and high levels of thioesterase activity. The remaining portion of the seeds evidencing these traits was employed to grow plants to form F3 seed. The selfing and screening and selection process is repeated to develop the fixed homozygous HSHO line, having the following fatty acid profile, C:16 5.4, C:18.0 24.8, C:18.1 58.5, C:18.2 7.2. Once the trait is fixed, similar HSHO lines can cross to form hybrid seed having both traits. According to the invention, sunflower plants and seeds from which said oil can be extracted have been obtained by means of a biotechnological process. This high stearic acid content is an inheritable trait and is fairly independent from the growing conditions.

Amend the paragraph beginning at page 27, line 11, as follows:

EXAMPLE 6 4

Plants growth conditions.

Sunflower (*Helianthus annuus* L.) seeds from high oleic lines used in example 5 3 with altered seed fatty acid content ~~was~~ were used to test for the thioesterase activities over stearoyl-ACP. Plants were cultivated in growth chambers at 25/15°C (day/night) temperature, 16 hours photoperiod and photon flux density of 300 micromol m-2s-1. Seeds for analysis were harvested at 15 days after flowering and kept at -20°C.

An abstract of the disclosure is provided on a separate sheet as new page 32.

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